Cell Reports, Volume *26*

Supplemental Information

Dlk1-Mediated Temporal Regulation of Notch Signaling Is Required for Differentiation of Alveolar Type II to Type I Cells during Repair

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SUPPLEMENTAL FIGURES

Finn et al Figure S1 ↑ up

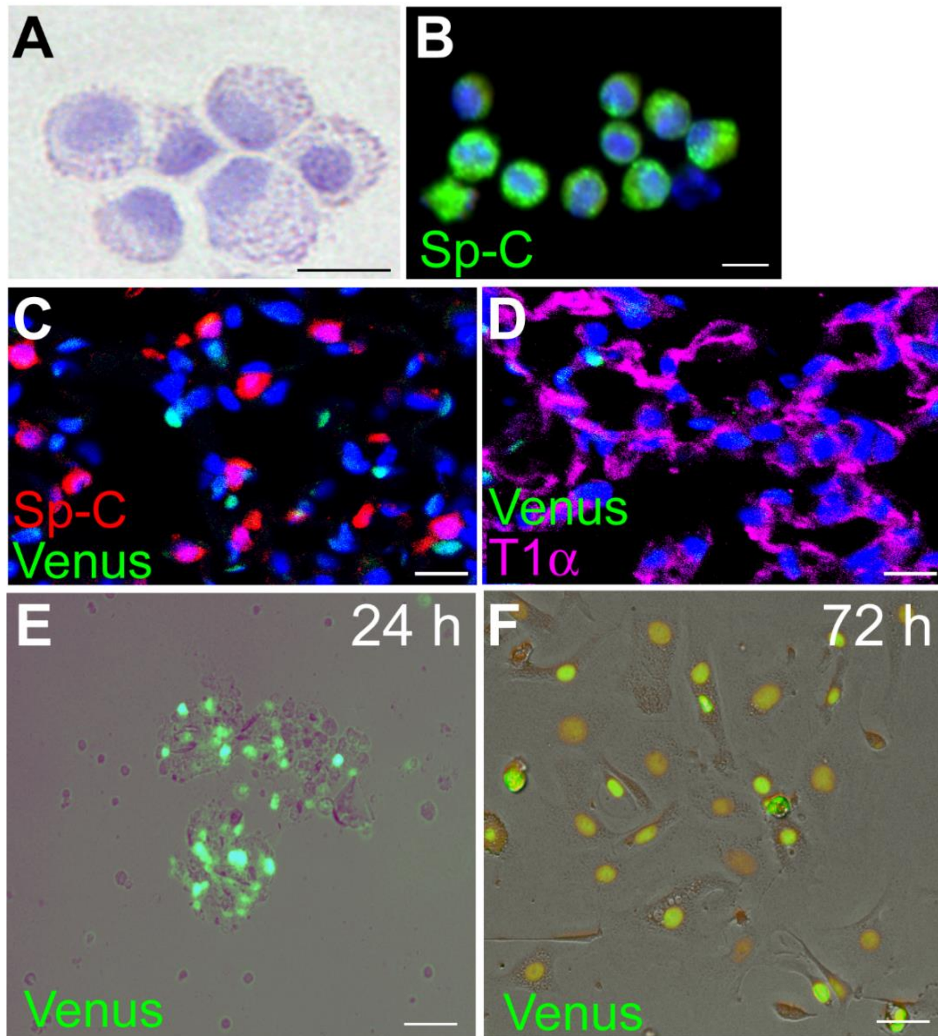

Figure S1 related to Figure 1. Culture of freshly isolated type II cells. (A) Papanicolaou stain showing most of the isolated cells having typical type II cell morphology. (B) Sp-C staining of freshly isolated type II cells is used to assess isolation purity. (C-D) Lung sections from non-PA *Notch-Venus* mice. Even though some cells showed Venus expression, most of the Sp-C expressing type II cells (C) and T1α expressing type I cells (D) were negative for Venus. (E-F) Freshly isolated type II cells from *Notch-Venus* mice were cultured on gelatin coated plates. At 24 h post culture initiation, only a few cells showed Venus expression (E) while almost all cells were Venus positive at 72 h (F). Scale bar=10 μm for A-B, 20 μm for C-D, 30 μm for E-F.

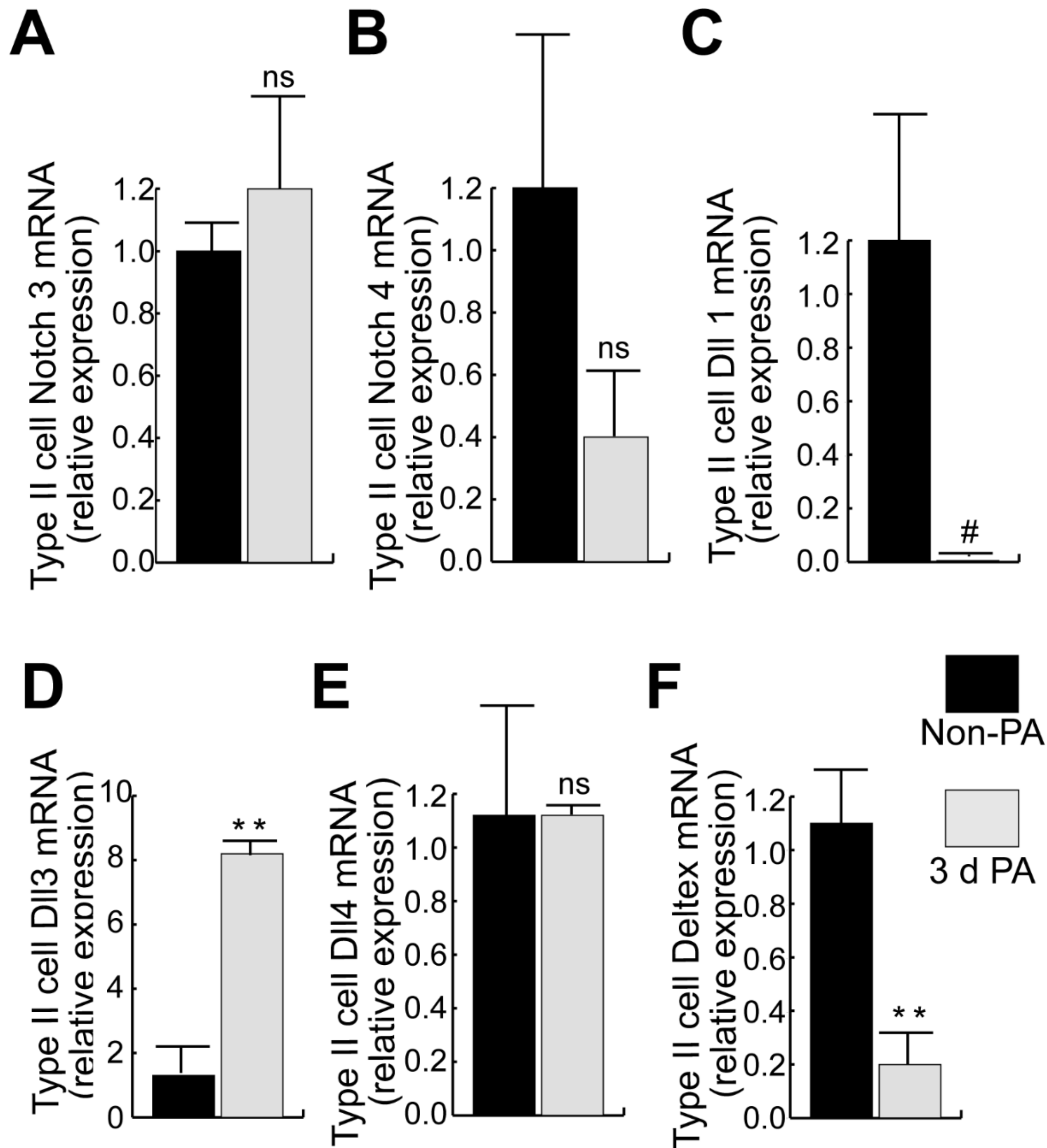

Figure S2 related to Figure 2. Assessment of Notch activation in type II cells at onset of repair 3 d post PA.

Real-time PCR analysis using type II cells isolated at 3 d after PA injury and from uninjured controls. (A) *Notch3*, (B) *Notch 4*, (C) *Dll1*, (D) *Dll3*, (E) *Dll4*, (F) *Deltex*. Mean±SE, n≥3. ns: p>0.1. #: p=0.075. **: p<0.01.

Finn et al Figure S3 ↑ up

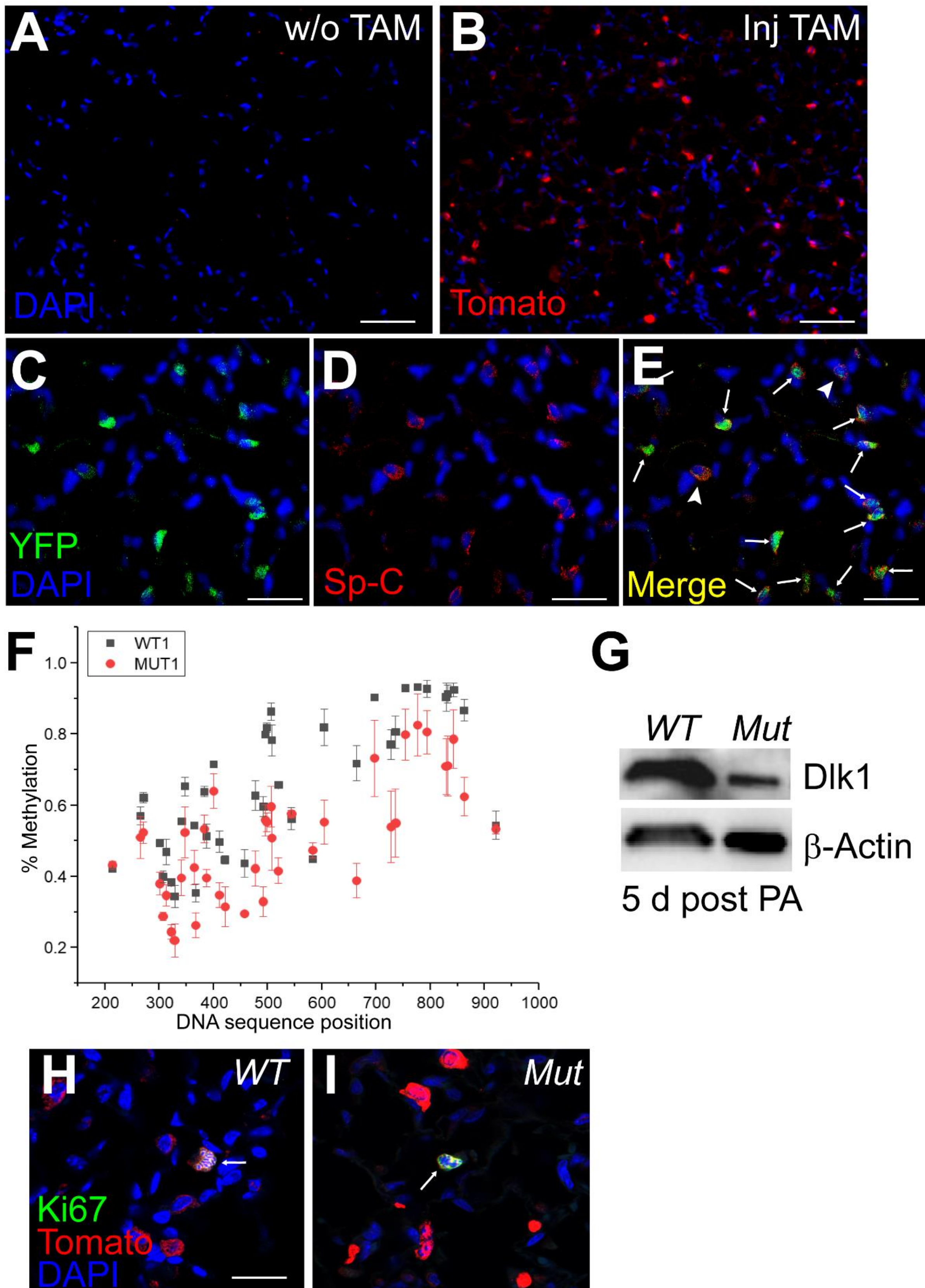

Figure S3 related to Figure 3. Type II cell specific disruption of Dlk1. (A-E) Specificity and efficiency of *SpC-CreER* driven lineage tracing and type II cell specific deletion. (A-B) Tomato and DAPI staining of lung sections from non-PA *SpC-CreER/Tomato* mice without (A) or with (B) tamoxifen (Tam) treatment. (C-E) were same section from a tamoxifen treated non-PA *SpC-CreER/+/ROSA-YFP* lung labeled by YFP (C), Sp-C (D), and merge (E). Arrow: nearly all the YFP^{+} cells were SpC^{+}. Arrow-head: only a few of $Sp\text{-}C^{+}$ cells were not labeled by YFP. (F) Methylation analysis of the last exon of Dlk1 using genomic DNA isolated from FACS sorted $Tomato^{+}$ type II cells of *WT* or mutant *(MUT)* mice. DNA methylation was assessed by bisulfite conversion followed by PCR amplification and sequencing. At each CpG site, counts of C and T were assessed, and percent methylation calculated by percentage of C out of total reads at each position. A comparison of the percent methylation between wild type and mutant were assessed based on two replicates for each group, and visualized in the software package Origin (2017; OriginLab). For almost all the positions, WT show a higher degree of methylation compared with mutant. (G) Type II cells isolated from Tam-treated *SpC-CreER/+/ROSA-Tomato (WT) or SpC-CreER/Dlk1loxP/ROSA-Tomato* ($Dlk1^{\Delta AT2}$ *or Mut*) mice at 5 d post PA were analyzed for Dlk1 expression by western blotting. β-actin was the loading control. Images are representative of 3 similar observations. (H-I) Lung sections were prepared from *WT* (H) *or* $Dlk1^{\Delta AT2}$ (*Mut*) (I) mice at 3 d post PA injection and processed for antibody staining against Ki67 (green) and Tomato (type II cell lineage marker). Scale bar=50 µm for A-B, 30 µm for C-E, 20 µm for H-I.

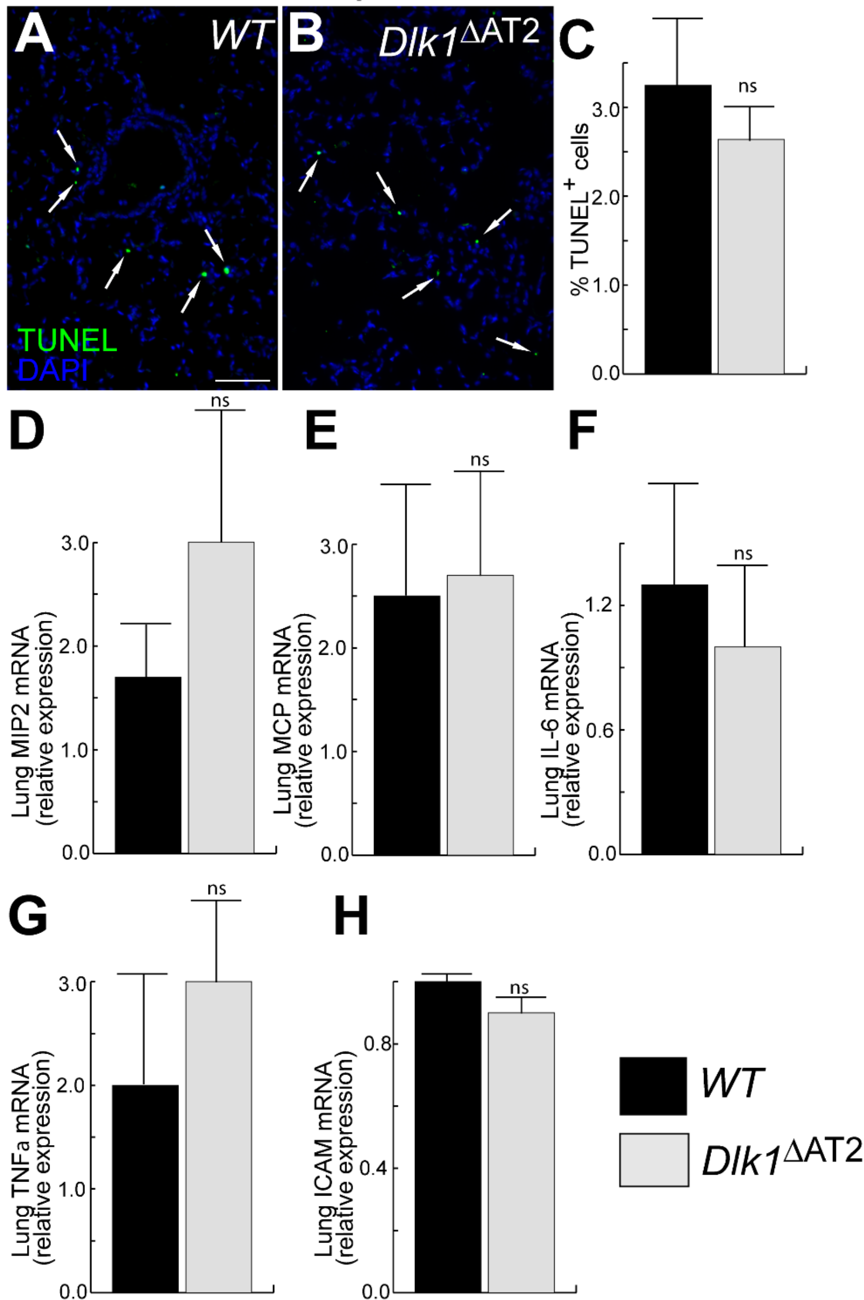

Figure S4 related to Figure 3. Cell death and inflammation phenotype of *Dlk1*$^{\Delta AT2}$ lungs. (A-C) Cell death was assessed by TUNEL staining of lungs isolated at 3 d PA from WT (A) and mutant (B) mice. Scale bar=50 µm. Numbers of TUNEL$^+$ cells versus all cells (DAPI$^+$) were scored for WT and Mutant lung sections. 500- 1000 cells were scored for each mouse, n=4 mice for each genotype. (C). (D-H) Lung lysates from WT and *Dlk1*$^{\Delta AT2}$ lungs were prepared at 3 d post PA and processed for qRT-PCR analysis for expression of genes involved in inflammation responses. n≥3 mice, mean±SE.

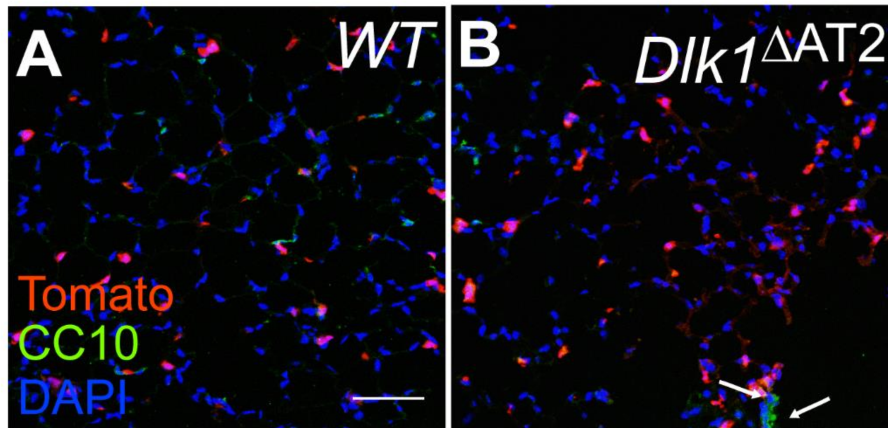

Figure S5 related to Figure 5. Type II cells from *Dlk1$^{\Delta AT2}$* lungs did not over-express club cell marker CC10. Cryosections of type II cell-lineage traced (Tomato^{+}) WT (A) and *Dlk1$^{\Delta AT2}$* (B) lungs were prepared at 7 d post PA and processed for antibody staining for club cell marker CC10. Arrows in B indicate CC10^{+} cells in bronchioles and bronchial alveolar ductal junction (BADJ). Scale bar=50 µm.

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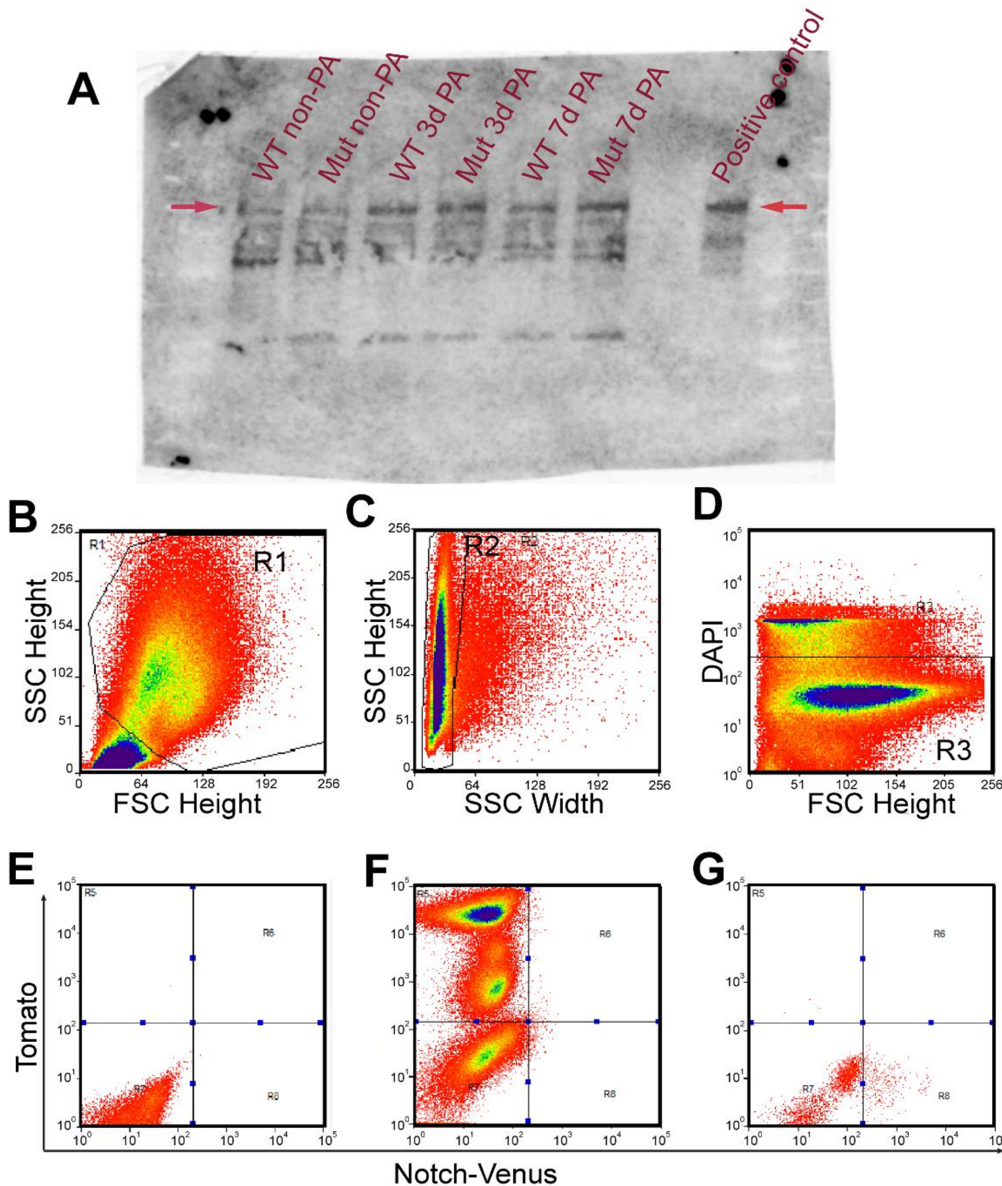

Figure S6 related to Figure 6. Notch activation in *WT* and *Mut* type II cells after PA injury. (A) Tomato$^+$ type II cells were isolated from uninjured, 3 d, 5 d and 7 d post-injury lineage labeled mice for western blot analysis of cleaved NICD. An original un-spliced blot is shown. Lanes are indicated, a positive control using mouse primary lung endothelial cell lysates was included. Arrows indicate NICD bands with correct size. (B-G) Controls for the FACS analysis in Figure 6. (B-C) Gates for forward and side scattering. (D) Dead cells were excluded. (E) Enriched type II cells from mice with no fluorescence labeling. (F) Enriched type II cells with Tomato only. (G) Enriched type II cells Notch-Venus only.

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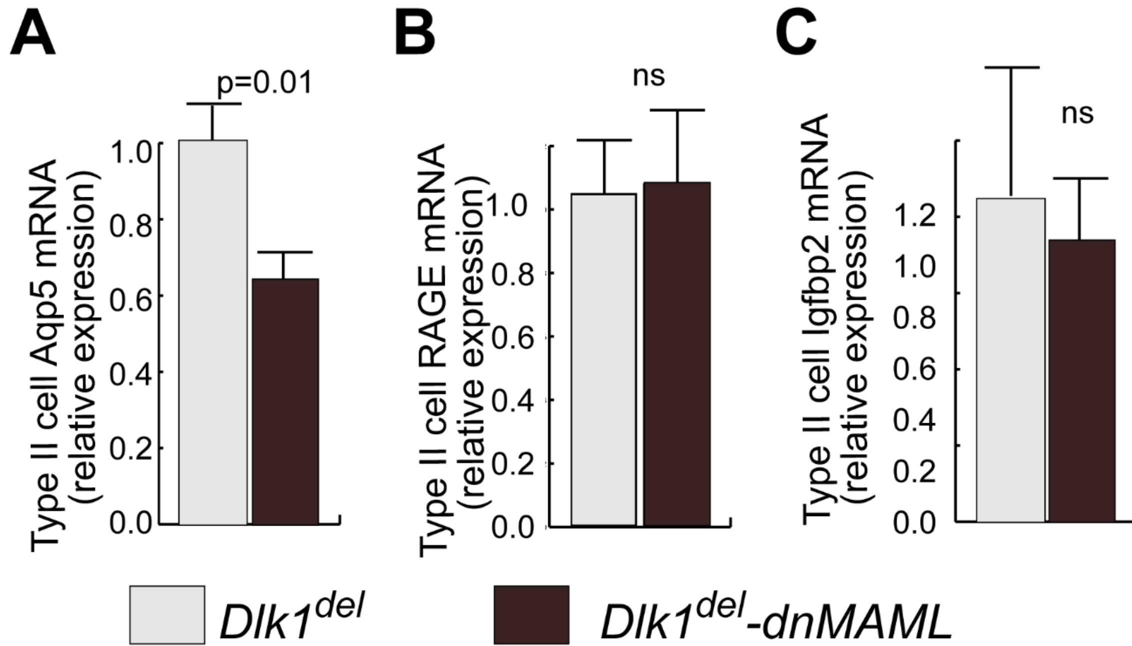

Figure S7 related to Figure 7. Partial rescue of type II cell *Dlk1^{del}* phenotype by *dnMAML*. Lineage labeled type II cells were isolated by flow cytometry sorting from *SpC-CreER/Dlk1loxP/ROSA-Tomato* mice *(Dlk1^{del}*) or from *SpC-CreER/Dlk1loxP/dnMAML* mice (*Dlk1^{del}-dnMAML*) at 9 d post PA and processed to qRT-PCR analysis for the expressions of type I cells markers.